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Short survey

The HIV/AIDS vaccine researchers' orientation to the process of preparing a US FDA application for an investigational new drug (IND): what it is all about and how you start by preparing for your pre-IND meeting

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1. Introduction

Despite numerous government publications, review articles and book chapters,[1] talks at conferences, and even an international association of "regulatory affairs" consultants[2] what to do to get permission from the US FDA[3] to test a new drug or vaccine remains a mystery to most academic scientists. Many investigators waste far too much time before contacting the FDA because they worry the requirements will be too onerous and others that do contact the FDA get sent a ton of documents (to "help" in filling out an IND application) most of which are unfortunately too long and tedious to be helpful or encouraging.

This article provides "user friendly" help to get HIV/AIDS vaccine scientists acquainted with the IND application process.[4] It is broken down into topical sections with a question and answer format, and much of the more technical information and asides[5] have been separated out into

[1] Some of these [1–3] are listed in bibliographic section with the Center for Biologics Evaluation and Research (CBER) and other website addresses [4–10] that give you all the government documents you need to refer to in filing an IND. You are especially encouraged to get and read the chapter by Donna Chandler, Loris McVittie and Jeanne Novak (Chapter 6: "IND application submissions for vaccines: perspectives of IND reviewers"), in Paoletti and McInnes' book, *Vaccines: From Concept to Clinic*, from CRC Press.

[2] The Regulatory Affairs Professionals Society (RAPS), http://www.raps.org/.

[3] But, is there an alternative to working through the FDA? Some small biotech companies (with the rationale that everything is being done inside one US State) have chosen to do some limited human trials under the aegis of their State regulatory board. They have hoped to quickly obtain some promising clinical data to facilitate further funding and speed eventual licensure. However, they have not really saved any time (this approach has even seriously delayed or killed development in some cases). Frankly, the state boards just do not have the expertise in manufacturing and clinical trials found at the FDA. Because the state boards do not hold the investigators to high enough standards, those investigators get lulled into thinking that they are taking a shortcut to performing studies that the FDA will accept. Only, later do they learn that their entire manufacturing process may need to be changed, or that all of the clinical studies they have done must be repeated with properly manufactured material or redesigned to get valid data. When they do finally come into the FDA with an IND application they may be back at square one with nothing to show for years of work! The message here is that you should not think of the FDA IND process as some troublesome obstacle and try to get around it. Rather, think of how you can use all the experience and expertise at the FDA to improve your manufacturing and product development processes.

[4] What does the author mean by the IND "process?" Process is a good word to use in order to keep in mind that this is not like an exam where someone (at the FDA) will give you a pass or a fail at some time and that is that; if you work with the FDA, they will work with you to help you through the process. The IND process is analogous to being back in grad school learning how to write a journal article. The process of writing that first paper was painful. Remember when you gave your first draft to your supervisor and every sentence got trashed. But, when you wrote your second paper you borrowed some of the first introduction, used the write-ups of the materials and methods that were the same, and followed the same format for results and discussion. It was easier the second time, and it got easier the more papers you wrote. Well, the big pharmaceutical companies have years of experience with IND applications. They have entire departments to assemble the product description, manufacturing processes, standard operating procedures (SOPs), lot-release tests, etc. into a familiar format. They plan all the necessary "IND-enabling" testing early in the pre-clinical stage of development; clearly, this shortens development time considerably, and is obviously easier than writing something from scratch. As an academic investigator, you do not have that alternative; you are working from scratch. The consolation is that, if you intend to stay in the vaccine field, getting your second IND will be much easier than the first (because now you will have a template to work from) and possibly more importantly, what you learn about product testing and manufacturing may refocus your thinking about vaccine design in very pragmatic and useful ways.

[5] How you should view the advice in this article? The author used to work for the FDA, so he knows about the IND process from the FDA perspective. If you are truly serious about getting a potential vaccine into clinical trials on your own (as opposed to through some large academic center or government clinical trials network, or with an experienced industry partner) you should use what is presented here only as an orientation, not as a complete do-it-yourself guide. You should get yourself a qualified regulatory affairs consultant; there are lots of these people out there and they have a professional organization that certifies them (see footnote 2).

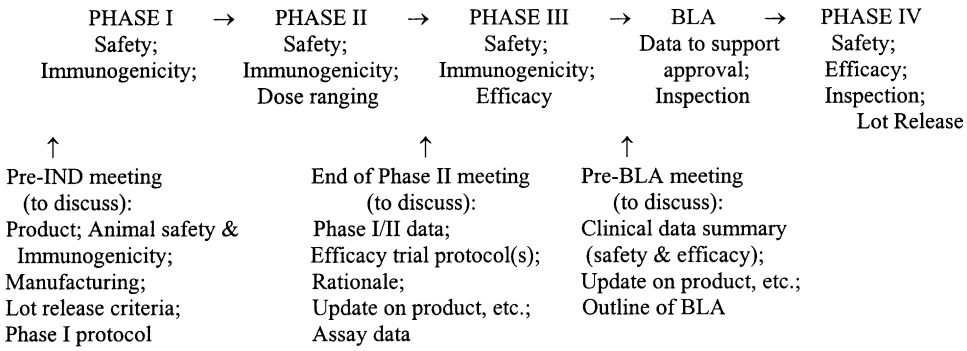

Fig. 1. Stages of clinical testing, review and regulation.

appendices and footnotes in an attempt to make it easier to digest. But enough introduction! The first and biggest mistake, that costs most investigators the most in development time, is putting off starting the process of getting an IND. So let us get to work.

2. The clinical testing stages of vaccine development

2.1. What are the critical stages in vaccine development as the regulators see it?

Look at Fig. 1. These are the stages of testing that your vaccine will have to go through before it can be licensed as a commercial product. This is a well-worn path; it has been developed over the course of years of development of many, many products. The "phases" refer to different levels of clinical trials (product testing in human beings). The shorthand description of these levels is that phase I is about demonstrating "safety", phase II is about demonstrating "activity", and phase III is about demonstrating "efficacy". Generally, phase I trials involve testing in tens of subjects, phase II in hundreds of subjects, and phase III in thousands of subjects.[6] In addition to the safety data (without which the FDA will not let you put your vaccine into larger numbers of people), there is information gained from each level of testing that is essential to setting up the next trial. The immunogenicity data from the phase I trial should guide you as you decide on the doses to test in phase II and the results of a good phase II dose-ranging study are absolutely essential to setting up a good efficacy trial.[7]

You should note that "safety" data is collected in all phases. To paraphrase the real estate agents' saying, the three basic laws of the FDA are: safety! safety! and safety! Now, you say that you know that your vaccine is safe. But many basically safe products have unanticipated side affects (any agent that is powerful enough to modify our physiology to get some desired affect is powerful enough to produce undesired side affects). The FDA is, first of all, a consumer protection organization.[8] Your main job in writing your IND application is to provide the FDA with the all data and information that they need to evaluate the potential safety of your vaccine. Take special care to present all the data you have that supports the safety of your product clearly and completely. Explain it fully in the text of your submissions (do not just refer to articles in your reference list). Have all the appropriate pre-clinical toxicology studies performed properly. If you convince the FDA people, from the very beginning, that you take their major concern (safety) seriously, then they will be much, much more helpful to you as you go through the rest of the process.[9]

You may also need a GMP (manufacturing) consultant. In addition, you should plan on using qualified contractors (contract research organizations (CROs)) to perform many of the steps in product development and production. Using CROs is called outsourcing; even large pharmaceutical companies are doing more and more of this in their own product development (and many of these consultants and contractors network through the Drug Information Association (DIA), http://www.diahome.org/).

[6] Sometimes you will hear people talk about phase I/II or phase II/III studies. These designations do not really make a lot of sense; often they are used as a compromise when the IND sponsor (i.e. you) thinks their product is further along in development than the FDA people think.

[7] It would be a tragedy to drop development of a potentially useful AIDS vaccine because the dose or immunization schedule was insufficient to generate what would have been an effective immune response if given one more time.

[8] It has seen major expansions in its regulatory powers in direct response to past food safety scares and drug safety disasters (i.e. in 1906 after Upton Sinclair's novel "The Jungle" frightened the public about conditions in the meatpacking industry; in 1938 following the death of more than 100 people from a poisonous "elixir of sulfanilamide", and in 1962 after Thalidomide deformed thousands of infants in Europe see http://vm.cfsan.fda.gov/~ird/history1.html for more detail).

[9] The flip side of "safety first" is that, at least in the IND process, other concerns, such as likelihood of efficacy, are less important to the FDA. Of course, demonstrating efficacy is absolutely essential for licensure, but up until that point the FDA people will bend over backwards to not make decisions on whether you can proceed based on their own personal thoughts about whether your vaccine will work. This presents a new and sometimes disorienting situation for you. The grant applications you have worked so hard to learn how to write have been judged on the sophistication of the science and whether the studies proposed are likely to be successful. Now a lot of you get upset when the FDA reviewers do

3. The pre-IND meeting

3.1. Must I fill out the IND application before I can start getting help from the FDA?

No. Your first real interaction with the FDA should be a pre-IND meeting. Notice in Fig. 1 that the pre-IND meeting is one of three meetings that the FDA expects will occur during the IND process (there will also be an end-of-phase II meeting and a pre-Biologics License Application (pre-BLA) meeting).[10] Please note that the FDA does not need this meeting to get information from you (you will be required to give them all the information they need to satisfy their regulatory concerns in your written IND submissions). This meeting is not a regulatory hurdle for you to pass (the FDA will take no actions based on this meeting). Rather, it should be viewed as a great opportunity for you to get specific advice from the FDA[11] before you proceed with costly and time-consuming development efforts.[12] The rest of this article is to help you prepare for this meeting.

3.2. How do I setup a pre-IND meeting for my vaccine?

HIV/AIDS vaccine developers request a pre-IND meeting by contacting the Division of Vaccines and Related Products Applications (DVRPA)[13] in the Office of Vaccines Research and Review, Center for Biologics Evaluation and Research (CBER). A telephone conference[14] call is then scheduled for within 60 days.[15] These pre-IND meetings used to be face-to-face, but with everyone's busy schedules and technical improvements in telephone conferencing they've been changed to telecons.

3.3. What information does the FDA want from me about my vaccine before the pre-IND meeting? When do they need it?

After the meeting is scheduled you should submit your pre-IND meeting materials so that they are received at least a month before the meeting.[16] You should prepare these

not appear to be impressed with the scientific beauty and likely efficacy of your vaccine; they ignore what you have written most proudly and instead wear you down with questions about safety. Well, they are actually working hard at appearing to be unimpressed; that is part of how they stay impartial—and the law requires regulators to be impartial. You will still have to convince the NIH reviewers (or private investors) of the possibility that your vaccine may work or you would not get the money to do the studies, but with the FDA concentrate on convincing them it is safe to try or you would not get permission to do the studies even if you have got the money. You see the FDA has evolved its procedures in response to regulating industrial sponsors, not academic investigators. Regulatory powers have not come easily to the FDA, and while industry has gradually acquiesced to all the safety regulations they will never allow "some government bureaucrats" to tell them ahead of time what will or would not work (and this is very appropriate—no amount of hypothesizing will ever substitute for just doing a controlled trial. We all know that some of the best experiments we have ever done were ones that other people told us would never work.). The attitude at the FDA has basically become "as long as it is safe, people are free to spend their own money on these studies even if we do not think it will work" and then they rely on some business sense and money limitations to rein in the less promising research. You will agree that this is far more pragmatic, realistic and fair than having somebody try to second-guess nature. So just satisfy their concerns about safety.

[10] Other meetings also may occur around specific problems. For example, you may request a meeting to resolve "clinical hold" issues, or the CBER vaccine people may have you present some non-routine issues to their external Advisory Committee at any time during the development process.

[11] If you have called or written the FDA (or cornered FDA people at meetings) you may have been frustrated by the difficulty of getting clear answers to questions of what will or will not be accepted. Many there would really like to help you more, but they can not for very good reasons. First of all, the law prohibits them from giving you too clear examples from previous INDs because you may recognize those products and that is all proprietary information. They guard proprietary information rigorously because if industry did not trust the FDA to keep information private industry would be less willing to disclose information to the FDA. Competent regulation would break down because there just is not enough money for the FDA to go out and search industry's books all the time—the system depends crucially on trust and confidentiality. Secondly, many of the products that the FDA (especially CBER, the Center for Biologics ...) regulates are quite novel. Their regulation frequently requires new decisions on what is acceptable, and the FDA is a "consensus" decision-making organization. Every IND application and amendment is read carefully by at least three people, and important questions are discussed by many more—and discussed, and discussed until a consensus is arrived at. The thing is, that until this process is gone through, no single individual knows what everyone else is going to say; so they really do not know whether a particular procedure or product will be acceptable or how long it will take to come to consensus (the guy down the hall may be a stickler on something no one else in the world is worried about; but he gets to put his word in). Everyone at the FDA is worried that, no matter how clearly they state that something is only their opinion, you will regard their "opinion" as a "promise" of approval from the FDA. If you feel frustrated now, imagine how you would feel if you spent millions of dollars to do something the way someone at the FDA told you it should be done and then submitted your IND, only to be put on hold because someone else at the FDA thought differently, and their opinion prevailed. At least when the FDA gets back to you with a consensus opinion on your submitted IND you can be relatively sure they would not change the rules on you later. Now before your pre-IND meeting the FDA will meet to develop consensus on your product as you present it in your submitted pre-IND meeting materials; this will allow them to be very specific in their responses. So the pre-IND meeting is actually your chance to get some clear answers from the FDA before going through all the work of putting together the IND application.

[12] Most sponsors plan the crucial "IND-enabling" safety and immunogenicity studies (discussed in Section 4 of Appendix A), but do not actually have them performed until they are discussed with CBER in the pre-IND meeting. In this way, they avoid having to repeat expensive batteries of GLP studies.

[13] 1 (301) 827–3070.

[14] In-person meetings can still be requested to discuss "clinical holds" and will be arranged for the other routine meetings.

[15] To understand how CBER schedules these meetings, see Section 3. Meeting Management Goals at their "performance goals and procedures", website: http://www.fda.gov/cber/genadmin/pdufago111297.htm.

[16] Usually, 10–15 FDA people will participate so you should submit 15 full copies of the meeting materials to be sure that every one of them will have a chance to read the material before the meeting. You want them all to have read your materials. Especially, you want them all to have read, thought about, and discussed your specific questions ahead of time (they will have a "pre-meeting" to hammer out consensus on important issues).

Table 1
Submission to the FDA: in requesting a pre-IND meeting

The pre-IND meeting materials will usually include:	
(1)	Meeting agenda
(2)	List of expected participants
(3)	Description of the product including scientific rationale and biochemical characterization
(4)	Summary of pre-clinical data with the proposed vaccine that support a clinical study including: (a) safety studies, and (b) activity studies (e.g. immunogenicity studies, neutralization assays, and investigations in animal protection models)
(5)	Previous human data relevant to the vaccine, if available
(6)	Summary of the manufacturing process including: a flowchart, a description of the manufacturing process, a description of the source and quality of starting materials, a description of in-process testing, and tentative lot-release specifications (description, identity, purity, sterility, general safety, potency)
(7)	Description of the manufacturing facility
(8)	Proposed phase I clinical protocol and the clinical development plan
(9)	List of questions or issues for discussion (e.g. formulation issues, toxicology study design, use of a novel adjuvant, adequacy of in-process or lot-release tests, trial design)

meeting materials carefully because this is what helps the FDA prepare to help you. Table 1 is a list of what to include in the pre-IND meeting materials you send to CBER before the meeting.

Your written pre-IND meeting materials will generally be 30–50 pages long. What to include in your written meeting materials is discussed in detail in Appendix A. You must read this appendix (especially, Section A.4.1 in Appendix A on pre-clinical safety studies which should help you understand how the FDA looks differently at different types of vaccines). But first there are a few basic rules that you should apply to writing your pre-IND meeting materials:

1. Do give them all of the information you want them to consider in the submission itself. Remember, these are busy people. They will read every page of IND submissions because they have to make decisions on those, but the pre-IND meeting is a favor to you. So make it easy for them to get the information you need discussed. Rarely do they have the time to go the 3–4 miles to the NIH campus library to look up references you cite for this meeting; many of them may not even read articles you attach. If you want something discussed put it in the main text of your submission, present it clearly, and ask a specific question about it.
2. Be clear, complete and concise. Include a figure or two (or even three—as many as are needed), even if you think all is clear from the text. Do not include a lot of unnecessary material like certificates of analysis, SOPs, sample Informed Consent Forms, or detailed subsidiary protocols. While all that material is required in the IND application, here it will only distract the CBER peoples' attention from the important product development issues you want discussed and discourage them from carefully reading the rest of what you submit.
3. Do not omit or hide (e.g. by burying it in a reference or "attached" document) supporting or adverse safety data. Remember safety is key. First impressions are frequently remembered better; absent supporting data may leave FDA reviewers with a vague feeling that there is some safety concern. It may take many IND amendment submissions for you to dispel these feelings. Absent adverse information may cause more delay than the "adverse" data itself; it will delay the design and execution of appropriate pre-clinical safety studies or may require clinical protocols to be put on hold while they are rewritten to examine new potential adverse events.
4. Prepare specific questions on everything you have doubts about (you should even ask about the acceptability of specific procedures, assays, protocols, etc. about which you are pretty sure). It is important to fill up the time of this meeting with questions to which you want the answers because this is your best opportunity to get input and information to facilitate the IND process for your vaccine.

4. Some critical issues in manufacturing

There are some important general issues to focus your attention on sooner rather than later. They are: (a) what is meant by the terms "GLP" and "GMP", (b) the importance of manufacturing consistency to reproducibility in biological products, and (c) concerns about "cell substrates".

4.1. What is meant by GLP?

Before you get into writing your pre-IND meeting materials you will already have done a lot of research. Much of what you have done is background and "proof of concept" work that the FDA does not need to see. However, in Section A.4 of Appendix A, there are some crucial pre-clinical studies that must be submitted, and these must be performed according to what the government defines as "Good Laboratory Practice"(GLP) (these "crucial" studies are the safety and activity studies discussed in Appendix A, Section A.4).

Then they can give you specific answers at your pre-IND meeting instead of saying "we will have to get back to you on that" (which is what they are likely to say if you suddenly raise new questions).

GLP is defined in 21 CFR §58.[17] GLP involves more rigorous record keeping than is standard in most research laboratories, so you really must read these regulations. Actually, it is frequently advisable to have these routine pre-clinical safety studies (e.g. systemic toxicology and local reactogenicity, sterility, cell line characterization, endotoxin level) performed by a contract laboratory—a Contract Research Organization (CRO)—that has performed many such studies, according to GLP standards, for submission to the FDA.

4.2. So then what is GMP?

Now, along with GLP you have probably also heard the abbreviation GMP; this is also defined by the CFR (21 CFR §211). When you examine the regulations on these two "practices" you will notice that GMP addresses much more procedural and mechanical detail than you ever think about in your own lab. While GMP, of course, is based on "good laboratory practice", it really addresses how large lots of product (lots large enough to be commercialized) are reproducibly made—this gets at the difference between experimenting and manufacturing.

From reading the regulations, you know that to get your vaccine licensed the FDA requires you to perform the supporting clinical trials on the precise product you want to license and sell, which means a product prepared by GMP. Many people worry that they must do even the basic pre-clinical safety and activity studies with GMP product. Thus, they think that getting an IND is going to require a prohibitive investment. This is not so. There are an increasing number of small companies (contract manufacturers) that are experienced in performing pilot scale GMP manufacture up to the standards of the FDA; you can contract with one of these companies to manufacture your vaccine and usually, for getting into phase I, many of the crucial pre-clinical studies (and sometimes all of them) can be performed with very good laboratory grade product,[18] although some of these studies will need to be repeated later when you have GMP production fully established.[19]

In some cases, the FDA may even allow you to do some limited phase I human studies with a very well characterized "almost GMP" product.[20] However, simple economics demands that only products made by GMP will get commercialized, so the FDA only wants to license GMP products, and thus that is what you must collect the ultimate safety and efficacy data on. But if you can demonstrate by lot-release tests that your "very good" laboratory-made vaccine prepared by the same (albeit scaled-down) process, is of comparable purity, and structurally the same as the GMP product then its "inherent" immunogenicity and any "inherent" toxicities should be the same. Thus, pre-clinical and early clinical studies of such product will be relevant and by "bridging" studies and in phases II and III studies you will collect enough safety and efficacy data on the GMP product for it to be licensed. Having said all this about using "very good" laboratory-made material for testing, you should not get the impression that everyone in CBER agrees. You may experience some "concern" from some CBER attendees at your pre-IND meeting for not working with GMP material. If you have the resources to set up or contract out GMP production, and especially if this is not your only vaccine (i.e. you are committed to becoming a vaccine company) then you should start GMP as early as possible and do the necessary "IND enabling" work with GMP product. But if you do not have the resources or backing, and especially if this AIDS vaccine is your one vaccine concept, then push for acceptance of your use of laboratory-made material in the early studies.

4.3. Why is manufacturing consistency so important for biologicals?

Pay very careful attention to writing Section A.6 in Appendix A. The FDA needs this information to know (a) that your product is what you say it is, (b) that it will be the same from lot to lot, and also (c) in order to further assess the safety of your product (adventitious agents and potentially toxic chemicals may be introduced during production

[17] Obtain from the Code of Federal Regulations, see reference [5].

[18] Confusion arises because many people (and, unfortunately, even some people within the FDA) do not understand the difference between laboratory-made and GMP product. Sometimes they think that GMP product is believed by the FDA to be somehow cleaner or safer. It is not. In fact, the FDA knows that very good laboratory-made product may sometimes be better quality than GMP product. The real difference between laboratory-made and GMP product is the difference between a Stanley Steamer and a Model T Ford. The Stanley was handcrafted by highly skilled workers and many people thought it was a much better car than the Ford which was made by unskilled laborers on an assembly line. But the Stanley brothers were unable to make enough cars at a low enough cost to supply even a small fraction of the need so they went out of business (and so probably will you if you attempt to supply the world with enough AIDS vaccine by whipping your post-docs to work harder!). Henry Ford was able to produce large numbers of vehicles cheaply with low-paid unskilled labor by establishing a factory production system where every step was systematized and regulated; production was divided up into finite small steps which required little training to perform, and which were always done precisely the same way. Well, similarly, laboratory-made product is prepared in the lab by PhDs one small lot at a time, while GMP product is made in large quantity by people who may not have even been to college (although most have; they just do not necessarily have advanced degrees) by following meticulously detailed protocols (standard operating procedures (SOPs)) in carefully designed workplaces with everything arranged (even the floor plan and air flow pattern!) to prevent mistakes. So while laboratory-made product "may" be better quality, GMP product can be made in much larger quantity and will be more reproducible. There is more assurance that every lot of GMP vaccine will work the same.

[19] This is to "bridge" your lab grade and GMP products, i.e. show that they act comparably. These bridging studies can be minimized with good records and good lot release tests.

[20] What time you save in getting into phase I by using this product may be lost later in the transition to a fully GMP-compliant product. However, you still may choose to take this risk if you are worried that animal immunogenicity studies are not good predictors of activity in humans and you need a quick answer to the human immunogenicity question before making or obtaining the investment in GMP production.

and knowing the process helps the FDA know what to look for). The safety reasons will be noted in more detail as we go through the information required, but a few words about the importance of the manufacturing process as it applies to the quality of "manufacturing consistency" are needed here. There are not clearly defined or agreed upon correlates of protection against HIV-1 which could be used to establish a totally reliable lot-release potency test. Thus, great reliance must be placed on "manufacturing consistency" to ensure that the commercialized vaccine will have the same efficacy as the tested lots. In the case of "biologics", it is, unfortunately, not sufficient to chemically test the final product to assure manufacturing consistency (although this is primarily what lot-release testing is about), as many small and difficult to detect differences may be introduced into complex biological molecules which may not show up in lot-release tests, but which may still affect immune responses.

4.4. What are these "cell substrates" issues that CBER is so concerned about?

Cell substrates are the living cells that your vaccine is produced in. The main point of all the cell substrate characterization discussion is to decide on the suitability of these cells for vaccine production—not just for their growth characteristics and stability in the manufacturing process, but most importantly from the standpoint of their freedom from adventitious agents (fungi, bacteria, mycoplasma, viruses, prions, etc.) and their low likelihood of transferring cancer promoting factors (oncogenic viruses, oncogenes, oncogenic proteins, unknown factors). The tests that CBER requires are briefly discussed in Appendix A (Section A.6), but the parenthetic lists in the preceding sentence should alert you to the root of the concern within CBER—how to test for "etc." adventitious agents [21] and "unknown factors" that may cause cancer. [22]

4.5. After the pre-IND meeting how much longer will it take before I get my vaccine into clinical trial?

It usually takes 6–12 months from the time of the pre-IND meeting to when an IND application is actually filed. This is because many investigators do not begin the critical pre-clinical safety and activity studies until clearing them with the FDA. Then those studies usually take 4–6 months to perform. Thorough preparation for the pre-IND meeting should focus you on the tasks to be performed and, most importantly, focus your specific questions to the FDA so that they can tell you exactly what remains to be done; this should shorten the additional time to starting your clinical trial.

Remember, this meeting will be your first real interaction with the FDA in the process of developing your vaccine concept into a real product. Keep in mind that it is a process; as was said earlier, product development is not like taking a test or a filling out a grant application that must meet some predetermined deadline. You will be required to examine your vaccine concept from new and different perspectives—the perspectives of product safety and manufacturing. While learning this can be a long process (it is hoped that this article will shorten it), it can also be an interesting process and you will learn a new body of knowledge that may also inform your future laboratory work. Do the best job you

[21] It will seem like an exorbitant amount of work is put into testing for "unknown" viruses. But if you recall the hullabaloo surrounding the contamination of early Salk polio vaccine stocks with SV40 and see the difficulty people recently had in disproving the hypothesis that the AIDS epidemic was the result of contamination of an early oral polio trial vaccine with SIV from chimpanzees, you will appreciate why the FDA is so cautious and, if you want to get really scared, think about the potential contamination of a vaccine, that goes into billions of people, with an undetectable agent that causes cancer or spongiform encephalitis 10–20 years later. Even with all the required tests as safeguards, people at CBER still worry that something they do not know about will get by them and cause a major new public health problem.

[22] While adventitious agent testing may seem onerous, at least it is mostly pretty straightforward and rational. By comparison, the discussion around the use of transformed cell lines frequently gets very confusing. On the one hand, it is clear that we will need to use immortalized cell lines to take full advantage of the new molecular techniques to produce the much needed large quantities of the much needed new vaccines. Furthermore, banks of continuous cell lines could be much more easily tested thoroughly for adventitious agents than large numbers of primary cell cultures—thus, vaccines made in them could potentially be safer. On the other hand, the use of such cells for vaccine production has been prohibited since 1954 due to the possible risk that it may "somehow" promote cancer in vaccinees. The problem for you, the would-be vaccine developer, if you want to (or must) use immortalized cells is that, in the absence of clear knowledge of that "somehow", the FDA is not really sure what to ask you to test for (they know to ask you to test for DNA—but it is not possible to make anything in a living cell and not have it contaminated with DNA! So with DNA the problem is that no one knows how much is safe—hence, the changing allowable level of extraneous DNA). Admittedly, this is a temporary problem that will be solved by understanding the specific mechanism of transformation of each immortalized cell line to be used, or by the construction of stable continuous diploid lines by specific molecularly defined non-transmissible means (e.g., cloning in telomerase, etc.). In fact, a recent meeting of CBERs Vaccines and Related Biological Products Advisory Committee (16–17 May 2001) suggested that the replication-defective adenovirus helper cell line, PER.C6, which was immortalized by transfection with a plasmid encoding the adenovirus E1 gene sequences by Fallaux et al. [Human Genet Ther 1998;9:1909–17] could be an acceptable substrate for production of adenovirus-vectored vaccines. However, this knowledge for other cell lines (or an acceptable cell substrate useful for production of your vaccine) could come in 2 years or not for 50 years. If you absolutely must use a transformed cell line you should consult CBER about it well before you submit your IND. You should understand that you may test it exhaustively with current technology and still not be allowed to use it to go into phase I trials in uninfected people of a prophylactic vaccine. You could try testing your vaccine as a therapeutic vaccine in people already infected with HIV (CBER does not worry as much about the "theoretical" possibility of causing a small number of tumors if a vaccine is going into a population of people already suffering from a life-threatening disease who are more willing to accept the relatively small potential risk). This will not overcome the FDAs concerns about tumorigenicity for a prophylactic vaccine, but at least you will not lose too much of the time you will need for developing your manufacturing process and product's safety profile while waiting for the technology and the FDA to catch up with the need (and an effective therapeutic AIDS vaccine would be useful too!).

Table 2
Submission to the FDA: IND content and format (original submission)

The IND must include (in the following order):	
(1)	Cover sheet (form 1571)
(2)	Table of contents
(3)	Introductory statement and general investigational plan: rationale and background; clinical development plan
(4)	[Reserved for future items]
(5)	Investigator's brochure: vaccine description and formulation; summary of pre-clinical and clinical safety, immunogenicity, activity data; risks and side effects
(6)	Protocol(s): (clinical studies)
(7)	Chemistry, manufacturing, and control information: vaccine characterization, manufacturing, and in-process/release testing; stability; environmental assessment
(8)	Pharmacology and toxicology information: vaccine safety/toxicity studies (in vitro or in vivo); immunogenicity; activity or efficacy in an animal model
(9)	Previous human experience: reactogenicity and immunogenicity for the same or similar products
(10)	Additional information

can with assembling your pre-IND meeting materials and do not worry too much about making mistakes at this stage because, as was also said earlier, the mistake that costs most investigators the most in development time is putting off starting the process.

4.6. What comes next?

Complete the necessary pre-clinical studies according to your discussion with the FDA in your pre-IND meeting, and assemble all the additional required documentation. Then you are ready to fill out ....

5. The IND application

A point-by-point discussion of all the actual IND content and format is too long for even this long introductory article. The contents and format of an IND application is described in detail in the CFR (21 CFR §312). The required sections in the IND application are listed in Table 2. Many of these sections will be just elaborations of sections you will have prepared for the pre-IND meeting materials, so you can at least start to work with the information you already have. Also, much helpful information for preparing your IND application is contained in the chapters by Mathieu and McInnes (Chapter 5), and by Chandler, McVittie, and Novak (Chapter 6) in [1].

But, here is some more very important advice for working with the FDA: always try to make it easy for the FDA reviewer to get the necessary information from your communications. Pay careful attention to pagination; every page of each submission should be numbered from the first page, in order, starting as page 001 (this i, ii, iii stuff in the introduction may look nice, but it just causes confusion). This is important so that later when you refer to some data you already presented (many submitted amendments earlier) you can cite the correct submission and page number and the reviewer will be able to find it. Speaking of citing information in earlier submissions (or cross-referenced INDs), please bear in mind that the reviewers do not have all your other submissions sitting on their desk; if they did then, since they are not just reviewing your vaccine, their offices would be so filled with documents there would not be room for them to sit, and they do not yet scan everything into the computer to have it available on an internal network. When you refer to something in an earlier submission they must request it from the Document Control Center and it frequently takes a week or more to get. This just delays a review that is important to you; keep such references to a minimum (except when necessary), and/or present the data completely again (especially if is brief) and note (clearly), where it was submitted earlier. Remember, if the reviewers at the FDA get confused or slowed down by anything it is only to your disadvantage.

But enough discussion. Go to Appendix A and get to work preparing your pre-IND meeting materials and even if you are not ready to start writing yet you should at least read through Appendix A, because there are a lot of testing and manufacturing pitfalls discussed that you really should know about, well ahead of time, to speed the development of your vaccine.

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Appendix A. (Pre-IND meeting materials)

This appendix contains more technical information to help you prepare your pre-IND meeting materials (to be submit-

ted at least 30 days before the meeting). It is organized to parallel the sections listed in Table 1 as information needed by CBER to evaluate your vaccine. The total pre-IND meeting materials will be about 30–50 pages long and should usually include the following sections (and appropriate subsections):

A.1. Meeting agenda

Usually, plan on about a 2 h meeting. The agenda should include short (15–30 min maximum!) presentations covering: description of the product (including scientific rationale and biochemical characterization), description of the manufacturing process, pre-clinical safety and activity data, lot release (and in-process) tests, and the phase I clinical trial. Do not waste time showing off new facilities or promoting your company. Focus on information about your vaccine, and be sure to allow time to get your questions answered.

A.2. List of expected participants

Make sure that the correct people, on your side, attend. Information gets garbled when passed through too many people; you want your crucial laboratory, manufacturing, QA/QC, regulatory affairs, and clinical people to hear exactly what the FDA has to say—directly from the mouths of the FDA. Because this is a telephone conference you are not limited in the number of people you can have listen in.

A.3. Description of the product including scientific rationale and biochemical characterization

This is one of the easier sections for an academic investigator to write because this is the sort of writing you do all the time. Write this as if you are writing it up for a journal article or a grant application; try to impress them that this is likely to actually work.[23] Also, as was said earlier, be clear, complete and concise. Include a figure or two (or even three—as many as are needed), even if you think it is all clear from the text (some people assimilate visual information more easily than text, and you do not want even one person in the room at the FDA to be confused). They absolutely need to know the precise nature of your product in order to evaluate the adequacy of proposed lot release tests. Also, this information may indicate potential toxicities that should be looked at in establishing safety in pre-clinical and clinical studies. If your product has evolved from earlier versions or other vaccines (especially, if there is some clinical safety or efficacy data on those other vaccines), you could discuss those here also, but make sure there is no confusion about what exactly the product is that you want to test now.

[23] While they would not waive safety concerns because of your arguments about expected efficacy, you are going to be working with these people for several years, if all goes well. It may help sometime, somehow for them to think your product has a chance of working.

A.4. Summary of pre-clinical data with the proposed vaccine that support a clinical study including: (a) safety studies, and (b) activity studies (e.g. immunogenicity studies, neutralization assays, and investigations in animal protection models)

If you have been paying attention up to now then you realize that this is a crucial section! It is also a very difficult section to give generalized advice on because many of the required studies are specific to different types of products. But do not worry too much, because if you miss anything the FDA will tell you so (that is after all, the reason for having this pre-IND meeting), and remember the IND process is not a test or a grant application that must meet some predetermined deadline. Both types of studies (safety and activity) are discussed, but first some general guidelines and definitions. All of these pre-clinical studies should be performed according to "Good Laboratory Practice" (as defined in 21 CFR §58, which you can obtain from [5].

A.4.1. Safety studies

Let us talk first about safety studies. There is routine safety (toxicology) testing that all products must go through, and there are more specialized studies required for some specific categories of products. Your basic "sub-unit" protein, peptide or particulate protein vaccine only requires the "routine" testing (unless, of course, you have mixed in some immunomodulatory molecule that should be tested for "immunotoxicity" or you have used some novel adjuvant, not contained in any licensed vaccines, that has to be toxicity tested on its own—hint, hint: life is easier if your vaccine works with alum or no adjuvant at all). For two of the specific categories: nucleic acid vaccines and virus-vectored vaccines (bacteria-vectored and eukaryotic cell-vectored vaccines share many of the same concerns as virus-vectored vaccines), there are specific guidance documents available from CBER that you should get and read (information on obtaining these documents follows, as also does some discussion of the special studies required for these types of vaccines). Other categories of vaccines under consideration for AIDS include "whole-killed" virus vaccines and pseudo-virion vaccines. There are no CBER documents that give detailed guidance on these types of AIDS vaccine, but the issues are pretty straightforward and will be discussed below. A last category of AIDS vaccine in development is the "live-attenuated" vaccine. Look, frankly, this completely scares the regulatory people at the FDA. But even they realize that it may come to this. Discussion has already begun within CBER about the sorts of pre-clinical safety tests that will be required for such a vaccine. However, that discussion is way beyond the scope of this simplified "self-help" article. If you are developing a "live-attenuated" HIV vaccine please contact CBER sooner rather than later for a list of suggested safety studies. Some of these studies may require a lot of time to perform. Starting them now rather than waiting for the failure of other vaccine modalities

before starting these necessary (but time-consuming) studies may save an enormous number of lives in the long run if a live-attenuated vaccine is all that will eventually work.

A.4.1.1. Routine toxicology. Routine toxicology is what is done even when no specific toxicity is expected (e.g. there are no chemicals or agents in your product that are known to cause cancer or destroy someone's liver, or no possibility of live viruses that cause a particular disease). One routine toxicity test, performed as part of lot-release testing to uncover toxic impurities introduced by mistakes in manufacturing, is the "general safety" test; this is very simple and will be discussed (see Section A.6 in Appendix A). More thorough toxicology is required at the pre-clinical stage to uncover toxicities that may be inherent to the product itself or any inescapable manufacturing impurities. To minimize "unexpected" adverse reactions occurring during clinical trials the FDA wants you beforehand to put at least the dose you intend to use in humans (and in the likely case that you may have to increase the dose to get a better immune response you should test at this time some multiple of your intended dose) into laboratory animals first (the basic dictum here is "do not use people as guinea pigs when you can use guinea pigs as guinea pigs!"). [24] These studies are usually performed on rabbits (because it is easy to get sufficient quantities of blood from rabbits repeatedly to follow serum chemistry and hematologic parameters). Three to six test rabbits (and controls) are inoculated with the vaccine (or adjuvant alone) one more time than the number of times you anticipate inoculating humans in clinical trials (just in case you find out during the phase I trial that you need one more boost). This can be done in an accelerated schedule to save time in pre-clinical testing, say every 3–4 weeks even if you plan on inoculating humans every 3–6 months. The animals are followed carefully for injection site reactions and for the development of any other adverse reactions by frequent clinical exam, serum chemistries and hematology, and they are observed for weight gain and food consumption. A couple of weeks after the last inoculation the animals are sacrificed and subjected to thorough necropsy examination for systemic toxicity (gross exam of organs and measurement of organ weights, plus histopathologic exam of immune system and hyper-perfused organs (bone marrow, spleen, lymph nodes, liver), and any organs demonstrating gross changes) and for "local reactogenicity" (histopathologic exam of the injection site). [25] You can use sera from these animals to demonstrate immunogenicity of your product by antibody production, but you will also need to do a more thorough immunogenicity or activity study (see Section A.4.2 in Appendix A) usually in mice, where it is cheaper to test more significant numbers of animals. Teratogenicity and tumorigenicity studies are not usually done at this stage; they are not required prior to phase I trials because those trials will be done in small numbers of people and almost always exclude pregnant women. However, if you do intend to vaccinate pregnant women in phase I, reproductive toxicology studies should be discussed with CBER.

A.4.1.2. DNA vaccines. Specific guidance on safety studies for plasmid DNA vaccines is contained in the CBER document "points to consider on plasmid DNA vaccines for preventive infectious disease indications" 22 December 1996—CBER document D0336. You can obtain this document by calling the CBER office of communication, training and manufacturers assistance (1-800-835-4709) or from [7]. These vaccines require additional safety testing primarily because the possible integration of vaccine DNA into the genome of vaccinated subjects may cause cancers by insertional mutagenesis (which may activate oncogenes or inactivate tumor suppressor factors). Thus, the FDA is asking sponsors of such vaccines to perform a pre-clinical study to address the integration potential of their vaccine. The FDA does not provide guidance for how this assay should be performed; however, two industrial sponsors have done everyone in the field a great service by publishing pre-clinical studies, of the potential for genomic integration of their DNA plasmids, that demonstrate levels of sensitivity acceptable (to CBER) for such a study. Those papers are: Nicols et al. [Ann NY Acad Sci 1995;772:30–9], and Martin et al. [Human Gene Ther 1999;10:759–68]. Use those papers as a guide to test your plasmid for somatic integration and you will probably be okay.

Another pre-clinical study that should be performed with DNA vaccines is a study of the distribution and localization (easily and most sensitively detectable by PCR or by detection of an encoded indicator molecule) to different tissues, and duration of expression of the plasmid. This should be performed on animals into which the vaccine has been inoculated by the intended route (e.g. subcutaneous, intradermal, intramuscular, intranasal) and intravenously (as a "worst case scenario"). Obviously, the results of such a study will guide you as to which organs should be examined in your potential integration study (discussed in the preceding paragraph). But, if there is unexpected or unusual localization

[24] "The dose" is defined as the actual human dose—not the human dose/kg. This is based on an important difference between vaccines and drugs. While drugs are distributed systemically and usually have their effects at a distance from the site of administration (e.g. the mouth), immunogens are presumed to act (at least initially) locally (at the injection site and in the draining lymph node). Important aspects of potential toxicity with vaccines may, thus, also be localized and dependent on the local injection site concentration which will be the same regardless the size of the rest of the animal.

[25] It is advisable to have the routine preclinical safety studies (e.g. systemic toxicology and local reactogenicity, sterility, cell line characterization, endotoxin level) performed by a contract laboratory (a CRO)—that has performed many such studies up to GLP standards for submission to the FDA. There are many contract animal facilities with qualified veterinarians that can perform the routine toxicologic studies for you.

or persistent expression, you may also need to perform longer-term studies to determine whether any pathologic immune (e.g. autoimmunity or tolerance induction) or local responses occur. Also, plasmid DNA vaccines should be evaluated specifically for potential of localization to the gonads (female and male) creating the danger of sexual or germline transmission and/or germline alterations. Lastly, tumorigenicity studies may be needed for some nucleic acid vaccines. This would be the case if your integration study suggests there is significant integration, if sequences of known oncogenic potential are present, or if your DNA vaccine contains extensive homology to sequences in the human genome. You will have to obtain specific guidance from the FDA on the conduct of such special studies. Some additional specific guidance for DNA vaccines can be found in Section VI.A. of the CBER document "guidance for industry: guidance for human somatic cell therapy and gene therapy" 30 March 1998—CBER document D0547. This document can also be obtained by calling the CBER office of communication, training and manufacturers assistance (1-800-835-4709), see [6]. If your vaccine generates "virus-like particles" they should be analyzed according to the advice given for pseudo-virion vaccines in next section.

Several laboratories are trying to enhance the immunogenicity of nucleic acid vaccines by adding molecular adjuvants: plasmids that encode immunostimulatory cytokines or co-stimulatory cell surface molecules. These plasmids should also be tested for biodistribution and integration potential. But, in addition, CBER is concerned about toxicity that may occur because of abnormal or persistent expression of the molecular adjuvant. Because these products are so new the FDA has not yet included any discussion of how to assess their safety in any guidance document. However, the first group to take such an adjuvant into a phase I clinical trial has thankfully published the studies that satisfied CBERs safety concerns; see Parker et al. [Gene Ther 2001;8:1011–23]. If you are planning to take such a molecular adjuvant approach use this article to plan your additional safety studies.

A.4.1.3. Virus- (and bacterial- or eukaryotic cell-) vectored vaccines. Specific guidance on virus-vectored vaccines can be found in the CBER document "guidance for industry: guidance for human somatic cell therapy and gene therapy" 30 March 1998—CBER document D0547. You can obtain this document by calling the CBER office of communication, training and manufacturers assistance (1-800-835-4709), see [6]. The specialized studies required for these types of vaccines will depend on whether your HIV immunogen is a sequence added into some non-pathogenic, but replication competent virus, such as vaccinia (or bacteria or eukaryotic cells, such as a non-pathogenic salmonella or yeast), or an HIV sequence that replaces vector virus genes to make a potentially pathogenic virus no longer able to replicate (e.g. as in the case of the alphavirus vectors). In the first case, you should demonstrate the non-pathogenicity of the vector, the stability of the non-pathogenic phenotype, and the communicability (ability to spread from vaccines to unvaccinated individuals) of the vector. All this should be easy to do from published studies if your vector is already a licensed attenuated virus vaccine; of course in that case the FDA will want history and sequence data that confirms the identity of your vector. In the second case, the FDA is worried about the pathogenicity of any vector virus that may be reconstituted as a result of recombination events during vaccine production. This concern can be addressed by using an attenuated virus as the vector backbone (of course, you must demonstrate its attenuation!). But there are not always animal models that adequately predict the human pathogenicity of viruses. Thus, you will also be required to test your vaccine for the presence of replication competent viruses (RCV), both in pre-clinical studies and as a routine in-process lot release requirement. There is a good discussion of detection of RCV in Section VI.C. of the "guidance for human somatic cell therapy" document. Such tests are critical to establish safety so they should be properly validated in your IND application (validation is the technical term for showing that your test is accurate, sensitive, specific, and reliable enough to be used to get the necessary information; validation can be tedious, but GMP requires validated testing—there will be more discussion, and references, on validation when manufacturing processes are discussed in Section A.6 in Appendix A). Additional concerns arise if your viral vector, even though it is not replication competent, can potentially (or intentionally) integrate into cellular DNA, such as some adenoviral or lentiviral vectors. In this case, the potential for tumorigenesis by insertional mutagenesis is present and you must address these issues as you would for a plasmid DNA vaccine (so get the CBER "points to consider" document mentioned in that "specialized safety" in Section A.4.1.2. Appendix A). But it would be a gross mistake to fail to point out that viral vectors that routinely integrate are very controversial as prophylactic vaccines within CBER (remember—prophylactic vaccines will go into millions or even billions of normal, healthy children, while the cancer vaccines and gene therapies these integrating vectors have been used for thus far, go into much smaller number of very ill people—the risk/benefit analysis is as different as night and day). If you go this route expect it may take the FDA a considerable time (if ever!) to come to consensus on letting you go ahead. Lastly, if the result of infection of cells by your virally-vectored vaccine is the production of HIV "virus-like particles" then you should also consider the advice given in the pseudo-virion vaccines, see Section A.4.1.5.

A.4.1.4. Whole-killed virus vaccines. The specialized testing required for "whole-killed" virus vaccines is very straightforward. You must show that the virus has been

killed, and CBER means killed! No one knows what the minimum inoculum of HIV-1 is that will infect a person, so CBER (to be on the safe side) is assuming it is one infectious virion. When a prophylactic vaccine gets licensed it will go into billions of uninfected, healthy people, and CBER absolutely does not want more of them to be infected by the vaccine than would be infected by the virus out there. There has been much discussion within CBER about what level of virus killing to require; the present consensus is that you should demonstrate that there is fewer than one infectious particle in a hundred million doses of your vaccine. Now you cannot show this by testing a percentage of each prepared lot for infectious virus because no test will detect a fraction of an infectious virus; if you tested 1000 doses and found no live virus then the best you could say is that there is fewer than one infectious virus in a thousand doses (not in a hundred million!) and you just cannot test a hundred million doses because, even if that were feasible which it clearly is not,[26] that quantity of material would physically swamp any assay so that it would never be sensitive enough to pick up one infectious virus. You also cannot validate that any single killing procedure kills enough virus to give this eight log safety factor because the FDA will only accept as much killing as you can demonstrate and nobody seems to be able to produce retroviral stocks with greater than 6–7 logs/ml titers of infectious virus. "What is to be done?" Follow this procedure:

a. First calculate how many infectious virions there would be in your vaccine if you did not use any virus killing techniques. There are two ways to go about this. You could: (a) measure how much p24 antigen there is per vaccine dose (i.e. μg/dose), then divide (a) by (b) 1 pg/infectious virion (this is the "to be on the safeside" estimate for how much p24 equates to the presence of one infectious virus particle from Krogstad et al. [AIDS Res Human Retrovir 1994;10:143–7) to get (c) the potential number of infectious viruses there are in one dose of vaccine.[27] This is a worst-case-estimate as it assumes that about one in a thousand particles is infectious, which people who try to make high titer lots will tell you is very good quality virus production. Alternatively, your virus harvest may not be such high quality (infectivity-wise) which is actually good because it means that your product may require less killing. In order to show that though you must seriously validate your virus titration assay so the FDA can rely on your numbers. Then: (a′) titer your culture harvest and multiply by harvest volume, (b′) determine your vaccine dose yield per culture harvest, and then divide (a′) by (b′) to get (c′) the potential number of infectious viruses per vaccine dose. Either way, if you now multiply (c) or (c′) by 100 million you will get how many logs of killing you must demonstrate (i.e. 14 $\log_{10}$ for a 1 μg p24/dose vaccine in the worst case scenario).
b. Now, you will probably need 14–16 $\log_{10}$ of killing, but you will only be able to validate 6–7 logs of killing with each procedure because that's the highest titer viral stock you will be able to test. Obviously you will probably have to combine two or three killing steps. There are several chemical, radiation, and physical methods for killing viruses that have already been studied, see Budowsky [Adv Vir Res 1991;39: 255–90 for a discussion of some of them) and new ones under study now (e.g. psoralens, heat, microwaves). No specific methods are mandated; it is your choice (taking into account parameters such as: cost, convenience, efficacy, and of course effects on immunogenicity). You may even choose to perform the same process twice (but note that this is different from doing it twice as long which does not necessarily give you twice the killing). But you must carefully validate your killing processes, independently, and then include routine manufacturing "in-process" controls and (validated) tests to document that the killing procedures have worked at each step for each lot. Also, you should note that the ability to perform the in-process tests might dictate the order in which you perform the killing techniques. For example, with a radioactive process you can position a sealed vial of live virus next to your vaccine batch in the irradiated container and then test that vial after to be sure that irradiation was successful, but chemical inactivation in separate containers may not be as comparable. Thus, you would perform a chemical inactivation on your culture harvest, where virus titration before and after of the harvest itself would confirm the success of the inactivation procedure. Then you would perform a radiation step on subsequent stages, where your assay probably would not be sensitive enough to detect the few live virus present at the start of the procedure anyway, but where you could verify the radiation killing with a control vial. Going into all the possible permutations of processes would make this discussion too long to be readable. Just keep in mind that there are a lot of things to think about here, but most of it is pretty logical. It would probably be advisable to consult with a manufacturing expert in this area and definitely be prepared to discuss all the intricacies of your inactivation procedure with CBER in your pre-IND meeting.

A.4.1.5. Pseudo-virion vaccines. In designing pseudo-virion vaccines, an attempt is made to construct a particle that shares many properties of the actual virus particle, without actually being a live virus particle, in the hope that the immune response(s) elicited by the pseudo-virion will be similar to that elicited by the virus, and thus, may be

[26] Your lot size would have to be more than a hundred million doses which is impractical and probably impossible, and if your vaccine were so cheap that it cost only one dollar a dose then this single lot release test would cost $ 100 million.

[27] To make this easy on you: if your vaccine contains 1 μg of p24 this calculates to be 10^6 infectious units; 10 μg of p24 equals 10^7 i.u., and so on.

effective against the live virus. You probably figure you want to get as close as you can to the live virus "line" without stepping over that "line". Well the FDA knows this so they want you to demonstrate that you have not overstepped by showing that no live viruses are formed during the production of your vaccine (or come out of cells transfected with a nucleic acid or vectored vaccine). As with "whole-killed" virus vaccines, the FDA wants to be sure that not one dose of vaccine in a hundred million (which is probably a gross underestimate of the number of doses of a successful HIV vaccine that will be delivered) includes live virus. Unfortunately, showing this is not as straightforward as validating killing in a "whole-killed" vaccine or demonstrating the absence of RCV from a "virus-vectored" vaccine. You see the FDA is not just worried that your vaccine will contain or generate live HIV-1 (which can be tested for), they are also worried that novel viruses may be activated or generated. One good way to ease their fears is if you can show that the pseudo-virion particles do not preferentially pickup "specific" nucleic acid sequences (i.e. especially, DNA or RNA that encode the proteins that make up the pseudo-virion). Unfortunately, the HIV gag protein contains a packaging signal that allows it to preferentially pick up HIV RNA by interaction with a specific nucleotide sequence in the nucleic acid; to be on the safe side you should probably mutate (deletion is preferable to site mutation because it cannot revert as easily) both the gag protein and the RNA. But even if there is no mechanism to enhance the inclusion of pseudo-virion protein encoding RNA it may be present in large quantities in particles that necessarily pick up random nucleic acid, simply because it is present in large quantity in the packaging cells. You will have to test your product for nucleic acid content in general and for the content of specific sequences. If specific sequences are present then there must be sufficient mutations to preclude the possibility of replication competent virus arising. You will also be asked to perform a couple of blind serial passages of your product on three different cell lines and then follow these cultures for microscopic evidence of cytopathic effects and test for hemadsorption as is recommended for the "routine" detection of unknown adventitious viruses in Section V.C.1. of the 1993 CBER document "points to consider in the characterization of cell lines used to produce biologicals". Another thing that the FDA wants to see is that there is no reverse transcriptase activity in the particles; this can be demonstrated by performing a PERT (pcr-enhanced reverse transcriptase) assay on your vaccine. If your pseudo-virion contains reverse transcriptase, by design, then it should be non-functional (and if inactivated by mutation then remember: deletions are more reliable than point mutations).

A.4.2. Activity studies

Now let us talk about activity studies. Activity can be demonstrated either as actual protection in an animal model, or as the ability to induce a particular immune response. Some investigators seem confused about whether they must show protection in monkeys before the FDA will let them begin phase I human trials. Look, there is still debate about how closely any of the non-human primate lentivirus models approximates HIV-1 infection and pathogenesis in humans. If there were a really well-accepted, good animal model in which to test HIV/AIDS vaccines then, of course, the FDA would require you to test your vaccine in that model and show that it gave some protection. However, there is not such a model system. The FDA does not require (or even recommend) you test AIDS vaccines in any specific monkey challenge model. Nevertheless, you may be encouraged to perform such a trial with your vaccine (or an SIV analogue vaccine) in order to get NIH funding (or even some large scale private funding) for development of your vaccine. Also, unfortunately, there is no agreement on the correlates of protective immunity for an efficacious HIV/AIDS vaccine. But the FDA does want to know that, in addition to being safe, your product actually has some effect that might reasonably be thought to contribute to protection against infection with HIV or delay progression towards AIDS. Furthermore, while the specific immune responses in animals you choose to test may not predict the exact human response (or even be relevant to what are eventually determined to be the real immunologic correlates of protection against AIDS), immunization of animals will yield valuable information on the dose and regimen (inoculation route, adjuvant, boosting) appropriate for clinical trials (practically speaking, the dosing information is the part of pre-clinical immunogenicity studies that CBER reviewers consider most important). Your activity studies may also give additional information on product safety. Furthermore, speaking of adjuvants, if you plan to use any adjuvant other than alum you must show that it contributes something to your product—that it actually enhances the activity of your immunogen.

All this is to say that it is probably best to perform a good dose ranging study in a fair number of mice (say 10 at each of 3–5 evenly spaced dose levels) so that you can plug your immune response result numbers into a Reed–Muench or Kärber statistical program to get an effective dose in 50% of the animals inoculated (ED_{50}) which will have some statistical significance. In this study you should look for the ability of your vaccine to induce the particular modality of immunity (neutralizing antibodies, CTL, T_{help}, specific cytokines, CD8 suppressor factor) that you hypothesize will protect. Remember, these data will be used to justify the doses proposed for the phase I human study; the study should be performed in accord with "GLP" and the results reported clearly. If you use any adjuvant other than alum, you should also test your immunogen alone and/or with alum to support the necessity of your adjuvant. You may also have to perform some limited immunogenicity studies in small numbers of non-human primates if there is some question about whether your vaccine modality works as well in all species (this has not been a problem when immunizing with proteins, but many nucleic acid vaccine constructs

have been much less immunogenic in primates than in mice).

A.5. Previous human data relevant to the vaccine, if available

While it is unlikely there is any previous human experience with your vaccine, there may have been some studies performed with closely related products (say for other diseases—e.g. the same DNA plasmid construct, but with an insert encoding a malaria or influenza antigen; or a different HIV-1 antigen in the same construct) or you may have already performed studies with your product without adjuvant or with a different adjuvant. Inclusion of summaries of safety, reactogenicity and immunogenicity data from such trials is appropriate, here, in your pre-IND materials. Obviously, the FDA reviewers will look at the data included here as a sneak preview of the potential safety and activity of your new product. So if there were any problems with the related vaccines they should be addressed in the design and/or testing of the new vaccine.

A.6. Summary of the manufacturing process (flowchart, description of the manufacturing process, description of the source and quality of starting materials, description of in-process testing, and tentative lot-release specifications, i.e. description, identity, purity, sterility, general safety, potency)

1. cell substrate testing
2. manufacturing processes
3. in-process testing
4. lot-release tests
5. potency tests
6. assay validation

For help in writing this section, you are strongly urged to read Paoletti's chapter "considerations in the production of vaccines for use in phase 1 clinical trials and preparation of the manufacturer's protocol" (Chapter 4 in [1]).

Start off with a complete and detailed, but clear and concise flow chart. Show each stage in product manufacture starting with your basic source material or plasmid, viral or bacterial seed. Indicate the processes and purification steps that take the product from stage to stage. Clearly, indicate, where in-process testing occurs and what that testing is. Take this diagram all the way through to release of filled vials (or syringes) and list the final lot-release tests. Remember this chart should clearly orient everyone to the overall process, so try to get it all onto one page because confusion may occur if people have to go back and forth between different pages (especially, if they are separated by pages of text).

Next, follow the flow chart with a narrative description of the manufacturing process for the specific lot(s) of vaccine intended for use in your phase I clinical trial. This will take three to five pages; it should be a detailed step-by-step description of the processes and purification steps involved in making your vaccine from the starting material (e.g. viral or bacterial seed, and working cell bank). The source and quality of all starting materials (including: viral and bacterial seeds, master and production cell banks, enzymes, sera, media, chromatography resins, adjuvant, etc. even water) should be described. In the actual IND you will not only have to describe the source and quality of materials, but also document it with certificates of analysis from qualified suppliers, but for the pre-IND meeting materials descriptions are enough.

A.6.1. Cell substrate testing

How cell banks should be characterized is described in the 1993 "points to consider in the characterization of cell lines used to produce biologicals". But what cell lines you can and cannot use is still a matter of debate within the FDA. It was the topic of an FDA-sponsored workshop, "evolving scientific and regulatory perspectives on cell substrates for vaccine development", 7–10 September 1999, and of recent meetings of the Vaccines and Related Biological Products Advisory committee to CBER (11–12 May 2000 and 16–17 May 2001).[28] The main point of all the cell substrate characterization required is to demonstrate the suitability of the cells to be used for vaccine production—not just for their growth characteristics and stability, but more importantly for their freedom from adventitious agents (fungi, bacteria, mycoplasma, viruses, prions, etc.) and low likelihood of transferring cancer promoting factors (oncogenic viruses, oncogenes, oncogenic proteins, unknown factors).

Testing for adventitious agents should include: (a) routine bacterial, mycoplasma (and spiroplasma for product made in insect cells), and fungal culture, (b) in vivo and cell culture inoculation tests for viruses, specialized tests for the presence of retroviruses, and (c) any other specific tests that are warranted, based on the passage history of the cell line, to detect specific possibly contaminating viruses.[29] Routine testing for bacteria and fungi is described in 21 CFR 610.12. Tests for mycoplasma are described in attachment #2 to the 1993 CBER "points to consider in the characterization of cell lines . . . ". Acceptable tests for spiroplasmas should be discussed with CBER. Because there is no test for the agent that causes BSE (bovine spongiform encephalitis—mad cow disease), in order to preclude its presence you are required to provide certification that all sera for cell culture come from herds known to be free of BSE (i.e. from non-BSE countries—so, not from Europe and especially not from the UK). Tests for adventitious viruses include specific PCR and immunofluorescence tests, as well as tests for

[28] Transcripts of the workshop are available at http://www.fda.gov/cber/minutes/workshop-min.htm and of the Advisory Committee meetings at http://www.fda.gov/ohrms/dockets/ac/acmenu.htm.

[29] E.G., viruses specific to the species the cells were derived from: bovine viruses if bovine serum was used; porcine viruses including porcine parvovirus if porcine trypsin was ever used.

unknown viruses.[30] Potentially contaminating murine retroviruses can be tested for by virus-specific infectivity tests, and unknown retroviruses can be tested for by the highly sensitive PCR-enhanced reverse transcriptase (PERT) assay. Most of these tests must be performed on the cells in the Master Cell Bank and on end-of-production cell cultures and, as it is for pre-clinical toxicology, it is usually easier and safer to have these tests performed by a contract laboratory that has experience performing them for the FDA.

Generally, CBER ensures the low likelihood of your vaccine transmitting cancer by prohibiting you from using transformed cells as a substrate for vaccine production. Recently, an exception was made for a cell line that was immortalized by the precisely defined transfection of two adenovirus genes.[31] Hopefully, more such specifically immortalized cell lines will be accepted for use in vaccine production in the future, but do not expect this to happen very rapidly. If there is any question about tumorigenicity you would still be wise to demonstrate that the cell line(s) you use are not tumorigenic. The tests for tumorigenicity are well described in the CBER "cell lines" points to consider document. You should test both the Master Cell Bank and post-production cells (cells from your working cell bank passaged in parallel to production cells at least until the end of the production process) for tumorigenicity in laboratory rodents (in vitro tests are not as reliable). These tests are, also, most appropriately performed by a contract laboratory.

A.6.2. Manufacturing processes

All processes should be described well enough that the FDA people can identify their appropriateness, limitations and any potential pitfalls. For example, do not just say "the immunogen will be purified from the harvest supernatant by affinity chromatography"—tell them exactly what the affinity matrix is (and what its source and quality is), how the immunogen will be eluted, what buffers and stabilizers are used, and what important impurities this is expected to remove. You should not include SOPs in the pre-IND meeting materials, but you must have written SOPs for all of your manufacturing procedures, even if you plan to go into phase I with laboratory-made material—thorough SOPs will facilitate the transition to GMP manufacturing.[32]

Lastly, describe the final formulation of your vaccine. Will the bulk be diluted or just aliquoted? Will the final product be lyophilized or in solution? Will it be combined with adjuvant during manufacturing or will a separate vial of adjuvant be provided to be mixed with the vaccine immediately prior to administration? Will it be in single-dose stoppered vials, multi-dose vials, or single-dose syringes?

A.6.3. In-process testing

In your flow chart and in your narrative description draw attention to any in-process testing. There are basically two different types of in-process tests that you could incorporate into the manufacturing process: (a) tests that ensure product safety, and (b) tests that monitor product consistency. Safety tests include tests for virus inactivation (remember, as discussed above under (a) safety studies, "whole-killed" virus vaccines, you cannot just test the final product for virus inactivation because then you are limited by the amount of product you test), tests for replication competent virus (RCV), or tests for the presence of adventitious agents (viruses) after culture procedures. You should check whether inactivation has worked or that no replication competent vector-virus or adventitious-virus is present or has been introduced, at the production stage(s) where this can be done most effectively and efficiently (and that is not necessarily in the final product!). Other in-process testing may be performed to establish or adjust reagent levels (e.g. determining protein concentration in order to set it to a predetermined optimal level before treatment with a chemical), determine yields of specific steps, or adjust product to final container fill specifications. You may designate such "yield" tests as "for information" while you are gaining experience with your manufacturing process. But, once you develop an understanding of what to expect when all goes well, then you should set lot-release specifications for such in-process tests. Such tests (especially, as they indicate efficiencies or yields of specific chemical or biologic processes or incubations) can be very informative of manufacturing consistency. For example, when your harvest yield is substantially lower than usual it indicates that something was different in the cell culture such that product was produced much less efficiently. The thing is that this may also indicate that a complex biological product may be molecularly different (e.g. differently glycosylated or otherwise post-translationally modified, differently folded or packaged) or contaminated with an adventitious agent difficult to detect with routine tests. Alternatively (and may be scarier), it may indicate that an error occurred in manufacturing (such as the introduction of a bad lot of a reagent, or a technical error made by a manufacturing worker); this should be investigated! You should probably exclude such

[30] You should perform inoculation into monolayer cultures of at least three cell types to be observed for cytopathic effects and hemadsorption after 4 weeks of culture; and also inoculation into eggs, suckling and adult mice, guinea pigs, and in some cases rabbits.

[31] The recommendation of the 16–17 May 2001 CBER Vaccines and Related Biological Products Advisory Committee meeting was to allow the use of adenovirus E1A/E1B transfected and immortalized PER.C6 cells in vaccine manufacture. Although immortalized these cells are only very poorly tumorigenic in animal models.

[32] SOPs are the written protocol instructions intended to guarantee that the product is made the same each time. GMP SOPs are usually extremely detailed and include spaces for recording source and lot numbers of reagents, and dates and signatures of person(s) performing tests; these are the instructions written to ensure and document that the technical people do the work properly with properly documented materials. It would not be inaccurate to say that the three basic laws of GMP production are document! document! document! The CFR sections on GLP (21 CFR 58) and GMP (21 CFR 211), and Paoletti's chapter discuss SOPs and list many of the procedures that require them.

harvests from your product unless you are absolutely sure you understand the underlying reason and effect.

A.6.4. Lot-release tests

Finally, you must detail the lot-release tests to be performed on your final product. In general, after the final steps have been performed in manufacture, but before the vaccine is bottled or put into single-dose syringes, certain "lot-release" tests will be performed on the "bulk product". Other "lot-release" tests (inspection and sampling of a pre-determined number of individual containers) may be performed on some of the final packaged product. The CFR covers the general biological products standard release tests in 21 CFR 610 which is available on the CBER IND website. Lot-release tests are often specific for each specific product, but they fall into the general categories of: Identity, purity, sterility, general safety, and potency. Identity tests will establish the identity of the vaccine product (i.e. make sure you have not made some disastrous processing error or mixed this product up with anything else being manufactured in the same facility); an immunoblot or ELISA for included antigens, or a restriction enzyme digest of a nucleic acid vaccine are common identity tests. Tests for purity of a vaccine include tests for pyrogenic substances and endotoxin content, and specific tests for removal of potentially toxic or dangerous components or contaminants introduced during manufacture (e.g. enzyme inhibitors, stabilizing agents, detergents, organic solvents, virus inactivating chemicals). Notice that the purity tests described here do not establish that nothing is present other than the precise components or antigens you intend (actually, in some vaccines, the specific virus or bacterial proteins may be only a few percent of the total), but rather just that toxic or harmful contaminants are not present. It is a good idea to get a Merck index and look up every chemical compound that contacts your product during its manufacture, and establish a lot release criterion for allowable residual levels of anything that has a toxicity. Vaccines manufactured in cells should also be tested for residual cellular DNA; the present recommendation is that residual DNA should be <10 ng/dose. There is also a regulation that vaccines should not contain more than 1 ppm serum (21 CFR 610.15(b)). However, given that serum is a complex mixture of many types of molecules that would be expected to fractionate differently in any vaccine's manufacturing purification process, no one at CBER knows how you should apply this regulation to lot-release testing (usually, people set a lot-release upper-limit specification for BSA and leave it at that).[33] Tests for sterility, viz. potential mycoplasma and bacterial contaminants are well described in 21 CFR 610. The General safety test is a required test for the detection of unknown extraneous toxic contaminants that involves injecting a set number of guinea pigs and mice with a set amount of product and watching them for 7 days basically to make sure they do not die. The reaction of most academic investigators upon hearing of this test is, "they want me to do what?" Look, this is a very crude, last-ditch test to pick up major mistakes; if anything ever failed this test you certainly would not want it to go into anyone's arm! Many of the routine purity (pyrogens, endotoxin, DNA), sterility and general safety tests can be performed by qualified contract laboratories that have experience performing such tests for the FDA. It may cost a little more to have the tests done by outside contract labs, but it simplifies and ensures your work enormously.

A.6.5. Potency testing

This brings us to potency tests. The CFR says, "tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in §600.3(s)". There the CFR interprets the word "potency" to mean "the specific ability or capacity of the product, . . . , to effect a given result". This is not super clear. In fact, most licensed vaccines are potency tested by checking their ability to elicit a specified titer of antibodies in laboratory animals. Thus CBER will allow you to use an antibody induction ELISA test to determine potency of your vaccine lots (you should probably set up a test, where you inject groups of 5–10 mice with decreasing doses of vaccine and then plug the results into a Reed–Muench equation to get an ED_{50} that you can compare to other lots). This is the case even when you do not expect that functional immunity will be based on antibodies. In cases, where clinical efficacy has been linked to pfu or cfu of viable attenuated organisms those measures have been used in lot-release potency testing. Similarly, CBER will allow plasmid DNA vaccines (and maybe some virus- or bacterial-vectored vaccines) to use a cell culture measure of "expression" inducing units for a potency test, even though antigen expression has not correlated well with immunogenicity in humans of DNA plasmid vaccines. Frankly, these are solutions to the problems of (a) the inadequacy of tests for cellular immunity (compared to the ease of performing and ability to standardize specific antibody tests), and (b) not knowing how to design a potency test in the absence of real knowledge of the specific "effect" needed to give protection against HIV/AIDS. As tests for functional aspects of cellular immunity evolve, and when more is known about actual immunologic correlates of protection for an HIV/AIDS vaccine, expect CBER to revisit this issue and require more meaningful potency tests.

A.6.6. Assay validation

Before we leave the discussion of manufacturing, please note that the FDA expects you to "quality" control your quality "control". By this is meant that you must demonstrate that the assays you use for in-process and lot-release testing are

[33] It may sometimes be simpler, as well as advisable from the standpoint of safety from unknown adventitious agent contamination, to eliminate serum from your cell culture medium altogether.

appropriately sensitive, specific and reliable; this is called "assay validation". It is especially important that assays that establish crucial safety parameters, such as virus inactivation or absence of replication competent virus (RCV), be validated. CBER issued a "guideline" document on assay validation way back in 1987 ("guideline on general principles of process validation" May 1987—CBER document D0063), and there is also an NIH publication on "validation and regulatory acceptance of toxicological test methods" that you could look at for help (the NIH document is actually available on the web at http://iccvam.niehs.nih.gov/doc1.doc). Assay validation is more involved than just doing an assay three times, taking the average, and determining the standard deviation. Actually, it is advisable to rely on approved commercial kits, experienced contract laboratories, and standardized tests already validated by other investigators as much as possible. You should not submit assay validation data in your pre-IND meeting materials, but it would not hurt to let the FDA know at this time that you are aware of the need to validate your assays and then you should submit assay validation in your IND.

A.7. Description of the manufacturing facility

This is pretty self-explanatory. In your IND application, you should provide actual layouts of any facilities used, a description of how product flows through the facility in the manufacturing process,[34] detailed equipment specifications, and qualifications of the crucial personnel. But for the pre-IND materials less elaborate, verbal descriptions will do (provided you give them enough information to determine that your product will be manufactured professionally, correctly, cleanly, competently and reproducibly). Briefly, describe the facility(ies) in which your vaccine will be manufactured. Where is the facility located? How large is it? How old is it? Who owns it? What specialized equipment is present? Is the facility dedicated to the manufacture of your product? If it is a multi-use facility, what other biologicals are/have been made in the facility?[35] Have any FDA licensed products or other products under IND been manufactured in this facility? Will the vaccine be entirely manufactured in your facility or will any parts (or all) of the manufacturing be performed by contractors elsewhere? Describe contractors' facilities, similarly. If contractors are making all or any parts of your product, are their facilities already licensed by the FDA? Are quality control/quality assurance procedures in place in the facility used? Are there personnel trained in GLP and GMP?

[34] Regulated product flow can be crucial in ensuring product identity and preventing contamination—in fact, experienced manufacturers frequently use building design to regulate product and personnel traffic as safeguards in product quality assurance. You will remember that Henry Ford's assembly lines moved only in one direction—not back and forth!

[35] A facility where spore-formers have been worked with is not suitable for production of any other biologics—ever!

A.8. Proposed phase I clinical protocol and the clinical development plan

The clinical protocol should describe the proposed human study, including:

i. hypothesis to be tested (the purpose of the study, the rationale—for a phase I study is usually "to evaluate the safety and immunogenicity of . . . "),
ii. study design, control groups, methods taken to minimize bias,
iii. number and characteristics of the subject population (i.e. the inclusion and exclusion criteria),
iv. vaccine dose(s) and regimen (number and schedule of boosts),
v. route of administration (IM, SQ, ID, IN, etc.), and
vi. methods for monitoring SAFETY (e.g. clinical exams, hematologic and blood chemistry studies, diary cards, telephone interviews) and immunogenicity (types of immune responses to be looked for and specific tests to be used) in human subjects.

Phase I studies usually involve tens of subjects (while phase II involve hundreds, and phase III involve thousands) and the primary endpoint as far as the FDA is concerned is safety. To satisfy legal concerns for protection of study subjects all studies performed under FDA IND must comply with the regulations governing "informed consent" (21 CFR 50, sub-part B) and IRB approval (21 CFR 56, sub-part A). Thus, CBER will require a copy of the IRB-approved informed consent form to be used, however that should be filed with the actual IND application and should not be included with the pre-IND materials (including detailed forms at this point will just distract from the discussion of basic issues). There is increasing discussion of ethics in clinical research; ethical human research involves much more than getting informed consent and an Institutional Review Board (IRB) approval. To orient yourself to these issues, it is strongly recommended that you read the recent JAMA article by Emanuel et al. [JAMA 2000;283:2701–11].

Earlier in this article, under the discussion of routine pre-clinical safety studies, it was pointed out that you are required to do routine safety testing, even when you do not expect problems. This is because the FDA would not allow you to "use humans as guinea pigs when you can use guinea pigs as guinea pigs". Well, having done those studies, now would not it be a good idea to use the results to make your human trials safer? You do not necessarily have to back off testing a potentially good vaccine because of mild toxicities or some negative physiologic effect that does not quite reach the level of toxicity. But do not ignore such findings! You can use the results from pre-clinical safety studies to improve the safety of clinical trials in two basic ways: (a) to establish stricter criteria for inclusion or exclusion of subjects in the study, and (b) to design clinical studies to watch

for the development of specific toxicities so they are detected before they threaten the health of your study subjects. For example, if you noticed a tendency for the hematocrit in your test rabbits to fall, although not to below normal limits within the time of your study, you might want to restrict phase I trial enrolment to subjects with normal hematocrits (42 ± 5 for women and 47 ± 5 for men) instead of the relaxed AVEG standard (≥ 34 for women and ≥ 38 for men) and then you should follow very carefully the hematocrits during the study and maybe even look at some additional parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular diameter (MCD) if hematocrits began to drop, even before subjects become clinically anemic, to identify any simple remedial measure. Above all, do not hide any adverse data from CBER! Especially, when you can use something to both make your study safer and demonstrate to the FDA that you take safety seriously too!

While CBER views the primary endpoint of a phase I trial to be safety, many investigators are more concerned about immunogenicity. It is practical, acceptable and even appropriate to address both sets of concerns with a limited dose-escalation or a dose-ranging study in phase I. If there is any possibility or indication (from pre-clinical studies) of toxicity, whatsoever, at higher doses then you should plan a dose-escalation rather than a dose-ranging study. In a proper dose-escalation study, the administration of increasing doses is staggered in time, and thus, the likelihood of any subject receiving a very toxic dose is reduced because lesser toxicity (or adequate immunogenicity!) would have shown up at the next lower dose. If there is a possibility that background activity may be confused with an immune response to the vaccine then you should consider including placebo controls and blinding your phase I study. Also, although it becomes much more important in phases II and III trials, you should start thinking now about using standardized, validated tests to evaluate the immune responses (relevant to what you hypothesize correlates with protection) that you expect from your vaccine. In addition, you should start to think about HIV diagnostic methods that will differentiate vaccinated individuals from those truly infected, because evaluation of efficacy later on will depend on accurate diagnosis.

If you plan on performing your clinical trials through the NIAID HIV Vaccine Trials Network (HVTN) then, please, tell CBER this (but still give them a description of the phase I trial as detailed above). They know that protocols for clinical trials performed through NIAID undergo exhaustive (and that word was chosen very appropriately) review before submission to CBER. If you will be using the HVTN, then they would not let the discussion of details of the clinical protocol detract from other important items in the pre-IND meeting. If you are not planning on using the HVTN then you must let CBER know about your access to clinical trials infrastructure and data analysis, and your general clinical development plan, in addition to clearly describing your phase I study. CBER expects that all IND clinical studies will be performed according to the standards of good clinical practice (GCP); many of these practices are discussed in the CFR section on the IND application (21 CFR 312), and in more detail in the International Conference on Harmonisation guidelines on GCP.[36] And if you are not planning on using the HVTN and you are not strongly connected into an established clinical trials site or network, then it is strongly suggested that you look into getting a Contract Research Organization (CRO) do your phase I study.[37] The FDA specifically allows you to transfer responsibility for performing such studies (as with other requirements of the IND process, such as pre-clinical studies and manufacturing) to CROs (in 21 CFR 312.52). It simplifies things enormously to hire competent, trained people to do things you do not have experience with, and it is far cheaper to have things done correctly the first time!

A.9. List of questions or issues for discussion (e.g. formulation issues, toxicology study design, use of a novel adjuvant, adequacy of in-process or lot-release tests, trial design)

If you do not ask enough specific questions to structure the meeting, or if you ask unfocused questions, this may allow some of the FDA attendants, who are less central to the decision making process for your product, to go off on tangents not critical to the development of your product. Also, specific questions will get more thoughtful attention than general questions like “any other concerns”? To get well thought-out specific answers you must ask specific questions, and you must have asked them sufficiently ahead of time for the FDA people to think out the answers and come to consensus.

Appendix B. (More “Food for Thought” on pre-IND meeting materials)

Dr. Shapiro’s Cheesecake[3,5] Recipe[6] (pre-IND meeting materials in development)[1,2,9]

Ingredients[4,6]

- 15–16 graham crackers, finely crumbled (one graham cracker is one square—not the two attached squares, which make a rectangle) (British “digestive biscuits” can be substituted for American “graham crackers”; you will need about 100 g of digestive biscuits)
- 1/4 cup (60 ml or 62.5 g) butter or margarine, melted
- 12 oz (340 g) cream cheese

[36] ICH Guidelines document E6: GCP, which is available from their website at http://www.ifpma.org/pdfifpma/e6.pdf.

[37] Many of these people network through ACRP, the Association of Clinical Research Professionals, http://www.acrpnet.org/index.html.

- 2 eggs, beaten
- 2 tea spoon (10 ml) lemon juice
- 3/4 cup (180 ml) sugar
- 2 tea spoon (10 ml) vanilla
- 1 cup (240 ml) sour cream (called "cultured cream" in some parts of the world)
- 3 and 1/2 table spoon (53 ml) sugar
- 1 tea spoon (5 ml) vanilla

Combine cracker crumbs and butter or margarine thoroughly. Pat into 9 in. (23 cm) pie pan.[6]

Combine cream cheese, eggs, lemon juice, sugar and vanilla. Beat[7] until light and frothy. Pour into graham cracker crust and bake in moderate oven (350 °F or 180 °C)[7] 45–50 min (remember to preheat the oven[6]). Blend sour cream with sugar and vanilla. Remove pie from oven and allow to cool for 5 min, then pour sour cream mixture over pie. Return to oven and bake 10 min longer. Place in refrigerator at least 5 h before serving (I usually make it the night before and then end up leaving it in the refrigerator for about 20 h before serving). Serves 10–12.[8]

If you want to try something slightly different you can substitute almond extract for vanilla extract.[6] You can also get fancy and top off your cheesecake with a layer of cherry, strawberry or blueberry pie filling.[5,6]

Enjoy![8]

PRE-IND meeting materials will contain the following sections (as they appear in Appendix A). The numerals used in superscript in the recipe correspond to the numbered section heading within this section.

1. Pre-IND meeting agenda

   I will plan on a 1.5–2 h meeting. The agenda will include short presentations covering: description of the product, description of the manufacturing process, pre-clinical safety and activity data, lot release test(s), and the phase I trial protocol. The meeting will be kept very friendly (the CBER personnel are people whose opinions about cheesecake I value); in fact one friend at CBER already suggested that my meeting would go better if samples were provided, but I am not clear on how to do this in a telephone conference. Although I anticipate a friendly meeting, I do not expect them to "exchange" recipes as I know they cannot show me what anyone else has under IND.
2. Expected pre-IND meeting participants

   My crucial personnel will attend (e.g. the actual cook and shopper, if I do not perform those tasks myself).
3. Description of product

   There is no product description in the recipe above, but in the pre-IND materials I will include a brief description of cheese cake as a dessert (at least enough so that no one at the FDA could mistake this cream cheese cake for a Gruyere cheese brioche) and the fact that it has two layers of filling (with an optional fruit-based overlay) in a graham cracker crust. It has good potential as the finale to a great dinner party. But the FDA should be alerted early on to its high caloric and fat content to evaluate pre-clinical safety studies and the plan for safety monitoring during human trials, as well as to decide on the need to apprise trial subjects (in the Informed Consent Form) of the health risks of rich desserts.
4. Summary of pre-clinical data (toxicology and activity)

   There are no specific toxicologic studies recommended for cheesecake. However, in routine toxicologic studies careful attention will be given to weight gain and pathologic evidence of arteriosclerosis. Activity studies may quantitate cheesecake consumption in the presence of other foods, as a measure of preference.
5. Relevant previous human data

   Data about the national consumption of cheesecake, preference for different recipes (and toppings) and commercial products, and profitability of restaurant cheesecakes may be included here. Also, CDC reports (if any exist) of bacterial contamination of cheesecakes should be included to help the FDA further predict the safety of this recipe.
6. Summary of the manufacturing process

   Here, will be included a flow chart and written description of the manufacturing process. The list of ingredients (including alternative almond extract and optional fruit pie filling toppings) may include some source information (e.g. brand names), but COAs will not be included in the pre-IND meeting materials (although they will be required in the IND).

   Measures are given in both the American (English) system and metric as an indication of the progress of the International Conference on Harmonisation (and the international applicability of cheesecake is as certain as the reasoning behind US FDA regulations). Furthermore, pending stability studies with different storage and transport conditions, it is not yet known whether global delivery will require local manufacture (which, outside the US, means in sites using the metric system).

   Since graham cracker crusts can be purchased pre-made in most supermarkets the preparation of the crust may be outsourced, to sites compliant with FDA regulations, to save time (the FDA allows you to transfer responsibility for steps in manufacturing to qualified CROs. If it is okay in vaccine manufacture why not in baking?).

   Remember to preheat the oven (oven pre-heating should be part of any baking SOP!). Licking out the bowls used for preparing the cream cheese and sour cream layers will be performed as part of in-process testing (in-process "tasting"). I usually perform lot release testing by trying the first slice as I serve the rest of the cheesecake to my guests; pre-dinner party testing will require preparation of multiple cheesecakes per lot or slicing the cake in the kitchen (out of view of the guests) to prevent the embarrassment that may occur upon bringing out a cake missing a slice.

7. Description of the manufacturing facilities

   A standard kitchen, having an oven and refrigerator with reliable thermostats, is necessary. Other equipment recommended is an electric appliance to beat the cream cheese/egg/lemon juice/vanilla/sugar mixture; this is much easier on the cook than a hand beater. The kitchen can be used to prepare other products, but SOPs should be in place for clean up and to ensure that incorrect ingredients (e.g. garlic salt, dishwashing liquid, cauliflower, broccoli) do not enter the cheesecake production stream.
8. Proposed phase I clinical trial/clinical development program

   Study participant inclusion criteria will be very broad. Previous experience with similar products suggests the FDA will probably allow inclusion of children and pregnant women in phase I trials of this cheesecake without requiring additional pre-clinical toxicologic studies, but a specific question on this will be asked at the pre-IND meeting. Obesity or elevated cholesterol could be exclusion criteria if full fat ingredients are used. However, preliminary studies have demonstrated that a healthier, lower fat, calorie and cholesterol version that tastes just as good can be made by substituting a cholesterol-free egg substitute for the whole eggs and lite (reduced fat) cream cheese and sour cream (but avoid the no-fat cream cheese and sour cream because they do not cook as well). Thus, using reduced fat ingredients would expand the includable study population. The size of each human trial will be limited by the lot size (each cheesecake serves 10–12). The oral route of administration will be used and boosts will be at the request of study participants. The incidence of requests for second servings will be used as a measure of product activity (enjoyment). However, while activity will be recorded in initial trials, it will be a secondary endpoint (the primary endpoint of a phase I trial is always safety). Unlike other clinical trials, retention and follow up in this study should be “a piece of cake”.
9. List of questions
   - 9.1. Are any “specialized” pre-clinical toxicologic studies required? Are teratogenicity or mutagenicity studies required before inclusion of pregnant women and children in phase I studies of this product?
   - 9.2. Is what is meant by “one graham cracker” adequately explained?
   - 9.3. If a “pre-made” commercial pie crust is used should lot release criteria be established for its quality, or will a COA from the manufacturer be sufficient?
   - 9.4. How frequently should the oven be calibrated?
   - 9.5. I plan on using a generic cream cheese instead of a major brand name (COAs will be provided). Is this acceptable?
   - 9.6. Is the use of artificial vanilla or almond extract acceptable? These can change the flavor. Would it be permissible to take both natural and artificial flavoring agents into human trials under one IND, or are separate filings required?
   - 9.7. It is intended to perform stability studies on refrigerated leftovers. Will this be acceptable, or does CBER also want to see stability studies at room temperature in case people leave the cheesecake out despite recommendations to store it in the refrigerator?

References

[1] Paoletti LC, McInnes PM. Vaccines: from concept to clinic. Boca Raton, FL: CRC Press, 1999.
[2] Mathieu M. Biologics development: a regulatory overview. Waltham, MA: Parexel, 1997.
[3] The biopharm guide to biopharmaceutical development. BioPharm, 1999. p. 8–38 (December Supplement).

FDA (and related) documents (and how to get them)

[4] CBER Investigational New Drug (IND) Application, website: http://www.fda.gov/cber/ind/ind.htm.
[5] Code of federal regulations, websites: http://www.access.gpo.gov/nara/cfr; http://www.access.gpo.gov/nara/cfr/cfr-table-search.html.
[6] CBER draft guidances, website: http://www.fda.gov/cber/guidelines.htm.
[7] CBER points to consider documents, website: http://www.fda.gov/cber/points.htm.
[8] How to obtain CBER documents by CBERs “fax-on-demand” or mail: http://www.fda.gov/cber/pubinfo/faxinfo.htm.
[9] International Conference on Harmonisation Guidelines, website: http://www.ifpma.org/ich5.html.
[10] CDERs orientation website for drug development: http://www.fda.gov/cder/regulatory/applications/default.htm.